# Evaluation of *Parthenium* Species for Resistance to *Verticillium dahliae*

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**ABSTRACT**

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Eight strains of *Verticillium dahliae*, six of which were isolated from hosts other than guayule (*Parthenium argentatum*), induced wilt in guayule. Diploid plants wilted faster after inoculation than tetraploid plants. Younger seedlings were more susceptible to infection than older ones. Guayule was more susceptible to infection by *V. dahliae* than were four other *Parthenium* species tested. *P. fruticosum* was susceptible to $S_{T-1}$, a cotton-defoliating strain, but tolerant to all other strains, including two isolated from guayule in the field. *P. tomentosum* var. *stramonium*, *P. incanum*, and *P. confertum* var. *lyratum* were tolerant to all the strains used.

Guayule (*Parthenium argentatum* Gray) is a rubber-producing shrub native to northeastern Mexico and southern Texas. Its potential as an economical, domestic source of natural rubber was investigated under the Emergency Rubber Project during World War II (6). Because of renewed interest in guayule as a source of rubber, a breeding program to increase rubber production on a per acre basis is under investigation (5).

Disease resistance is a contributing factor to high rubber yield. An important disease of guayule encountered during the Emergency Rubber Project was Verticillium wilt (4), which was first observed in guayule in 1942 at Salinas, CA (2). Schneider (10) conducted a detailed survey from 1943 to 1945 that indicated widespread, heavy infection of guayule by *Verticillium* in Kern County, CA. He did not, however, identify the species. In some fields, 75–100% of the plants were infected (9).

The planting of guayule on land previously planted with susceptible crops (eg, cotton, mint, peppers, potatoes, tomatoes) and irrigation may increase the incidence and intensity of Verticillium wilt in the field (10). Guayule cultivars with field resistance to Verticillium wilt should be developed for future plantings. We studied the reactions of several *Parthenium* species to different strains of *V. dahliae*.

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## MATERIALS AND METHODS

***Parthenium* species.** The genus *Parthenium*, a member of the Compositae, comprises 16 recognized species in four sections (8). Guayule originated in eastern Durango, Mexico, the only region where sexual diploid forms were found (7). Many guayule cultivars were developed during the Emergency Rubber Project (4). The LA-1 line, used in some of our studies, comes from the USDA commercial cultivar 593. Our diploid line (75225) comes from the National Seed Storage Laboratory, Fort Collins, CO. Other species of *Parthenium* are from the Rollin's collection (8).

***V. dahliae* strains.** Several strains of *V. dahliae* Kleb. were obtained for our study. $C_1$ and $C_2$ are local isolates from guayule plants that were grown in a test plot at Whittier, CA. We obtained a cotton-defoliating ($E_{V3H}$) and a nondefoliating ($E_{330}$) strain from D. C. Erwin, University of California, Riverside; a cotton-defoliating ($S_{T-1}$) and a nondefoliating ($S_{SS-4}$) strain from W. C. Schnathorst, University of California, Davis; a local strain (F) from the Brazilian pepper tree from Peter Fliegel, Agricultural Commissioner's Office, Los Angeles County; and another local strain (M) from Martin Stoner, California State Polytechnic University, Pomona.

**Inoculum.** Isolates of *V. dahliae* were grown in yeast extract broth (4 g of Difco yeast extract, 20 g of glucose, 1 g of $K_2HPO_4$, 0.5 g of $MgSO_4 \cdot 7H_2O$ in 1 L of distilled water) on a rotating shaker (150 rpm) for 3–5 days under dim daylight and dark night. Spore suspensions were calibrated to a range of $1 \times 10^7$–$5 \times 10^7$ spores per milliliter for inoculation by soil drenching. Difco potato-dextrose agar and prune extract agar were used for culture differentiation. The prune extract agar was made by boiling 5 g of dried prunes for 30 min in 1 L of water. After straining, 5 g of lactose, 1 g of Difco yeast extract, and 25 g of agar were added.

**Soil drench procedure.** Seedlings 4 to 5 mo old were used for inoculation assays. They were grown in 4-in. plastic pots containing steam-sterilized UC-C Mix (University of California at Riverside) containing 50% peat and 50% silty sand. Glass tubing (0.9 mm OD) was used to punch four holes about 3 cm deep around a potted seedling. One milliliter of spore suspension (at $5 \times 10^7$ spores per milliliter) was delivered into each hole with an Eppendorf pipette, and was then covered with soil. To compare disease severity under different conditions of inoculation, we also used 0.1, 0.25, and 0.5 ml of spore suspension per hole. Hypodermic injection was another method of inoculation used for comparison (1).

The inoculated seedlings were then incubated in a growth chamber at diurnal temperatures with settings of 24 C for 14 hr of light and of 18 C for 10 hr of darkness.

**Wilt index.** Resistance of the plants to *V. dahliae* isolates was recorded by degree of wilting, with 0 = no wilt, 1 = 5–25% wilt, 2 = 25–50% wilt, 3 = 50–75% wilt, 4 = 75–95% wilt, and 5 = dead. Wilt symptoms began in side branches from

**Table 1.** Effect of inoculum amount on wilt severity in guayule line LA-1 inoculated with *Verticillium dahliae* strain $E_{V3H}$

| Inoculum[a] | Wilt index[b] after 23 days | 29 days | 36 days | 44 days |
|---|---|---|---|---|
| 0.4 ml/pot | 0.6 ± 0.37[c] | 1.4 ± 0.49 | 1.8 ± 0.75 | 2.4 ± 0.49 |
| 1.0 ml/pot | 0.9 ± 0.92 | 2.0 ± 0.63 | 2.5 ± 0.89 | 2.6 ± 0.49 |
| 2.0 ml/pot | 1.2 ± 0.40 | 2.2 ± 0.75 | 2.6 ± 0.49 | 2.8 ± 0.75 |
| 4.0 ml/pot | 1.8 ± 0.98 | 2.2 ± 0.75 | 2.6 ± 0.49 | 2.8 ± 0.40 |
| Hypodermic injection | 0.9 ± 0.20 | 1.6 ± 0.49 | 2.0 ± 0.63 | 2.2 ± 0.75 |
| Control[d] | 0.4 ± 0.37 | 0.6 ± 0.49 | 0.6 ± 0.49 | 0.8 ± 0.40 |

[a] Spore suspension at $5 \times 10^7$ spores per milliliter.
[b] 0 = no wilt, 1 = 5–25% wilt, 2 = 25–50% wilt, 3 = 50–75% wilt, 4 = 75–95% wilt, and 5 = dead.
[c] Each value is presented as the mean of five replicates with standard deviation.
[d] Four milliliters of water instead of spore suspension was delivered into punched holes.

the bottom and gradually progressed toward the stem tip.

## RESULTS

**Amount of inoculum.** Our comparison of disease severity under different conditions of inoculation indicated a slight increase in the wilt index as more spores were added per pot (Table 1). Spore amounts varied from 0.4 to 4.0 ml/pot. However, the differences were more pronounced at early stages of infection. Forty-four days after inoculation, plants treated with different spore amounts showed little difference in the wilt index (Table 1). Therefore, seedling resistance was not greatly affected by varying amounts of inoculum within the range indicated and at a concentration of $5 \times 10^7$ spores per milliliter.

Results obtained by hypodermic injection were comparable to those obtained by root drenching, but it was impossible to inject standard amounts of spore suspension into each woody seedling.

**Strains of *V. dahliae*.** A diploid line and the tetraploid LA-1 line of guayule were inoculated in two separate experiments with each of the eight strains of *V. dahliae*. Wilt developed 7–10 days earlier in the diploid than in the tetraploid seedlings (Table 2), which began to wilt in the third or fourth week after inoculation. The different strains of *Verticillium* produced more variation in wilting during early symptom development than when the disease reached its terminal stage.

Based on early observations, the most severe wilting of diploid guayule was induced by $C_2$ and $E_{V3H}$. The most virulent to the LA-1 line were $C_2$ (Fig. 1) and $S_{T-1}$. M. strain was particularly mild on LA-1.

**Age of seedlings.** LA-1 plants aged 2, 3, 5, 7, 8, and 18 mo were inoculated with the $C_2$ strain of *V. dahliae* with 10 replicates per age group. About 15 days after inoculation, wilt symptoms were observed in 2-mo-old seedlings; at 19 days, wilt appeared in 3-mo-old seedlings. Higher wilt scores were invariably obtained from the younger seedlings, which eventually succumbed to the disease (Table 3). Older plants were comparatively resistant to *Verticillium*. The young seedlings (2 and 3 mo old) were all dead 44 days after inoculation, while the oldest plants (18 mo old) showed no wilt symptoms.

**Species of *Parthenium*.** *P. fruticosum*, *P. tomentosum* var. *stramonium*, *P. confertum* var. *lyratum*, *P. incanum*, and *P. argentatum* (guayule) were separately inoculated with the eight strains of *V. dahliae*. Forty days after incubation the only species besides guayule that developed severe wilt was *P. fruticosum*, which was inoculated with $S_{T-1}$ (Fig. 2). $C_1$ and $C_2$, both isolated from naturally infected guayule plants, did not induce wilt in *P. fruticosum*. The pathogen was reisolated from all inoculated species of *Parthenium*. The six strains of *V. dahliae* that were used were recovered from all of the wilted guayule. $S_{T-1}$ was also recovered from all wilted *P. fruticosum* plants (Fig. 2). However, one of five recoveries was positive from symptomless *P. fruticosum* plants inoculated with $C_1$ and $E_{V3H}$. One positive recovery was also obtained from a symptomless plant of *P. incanum* that had been inoculated with $S_{T-1}$. F strain was reisolated in a single

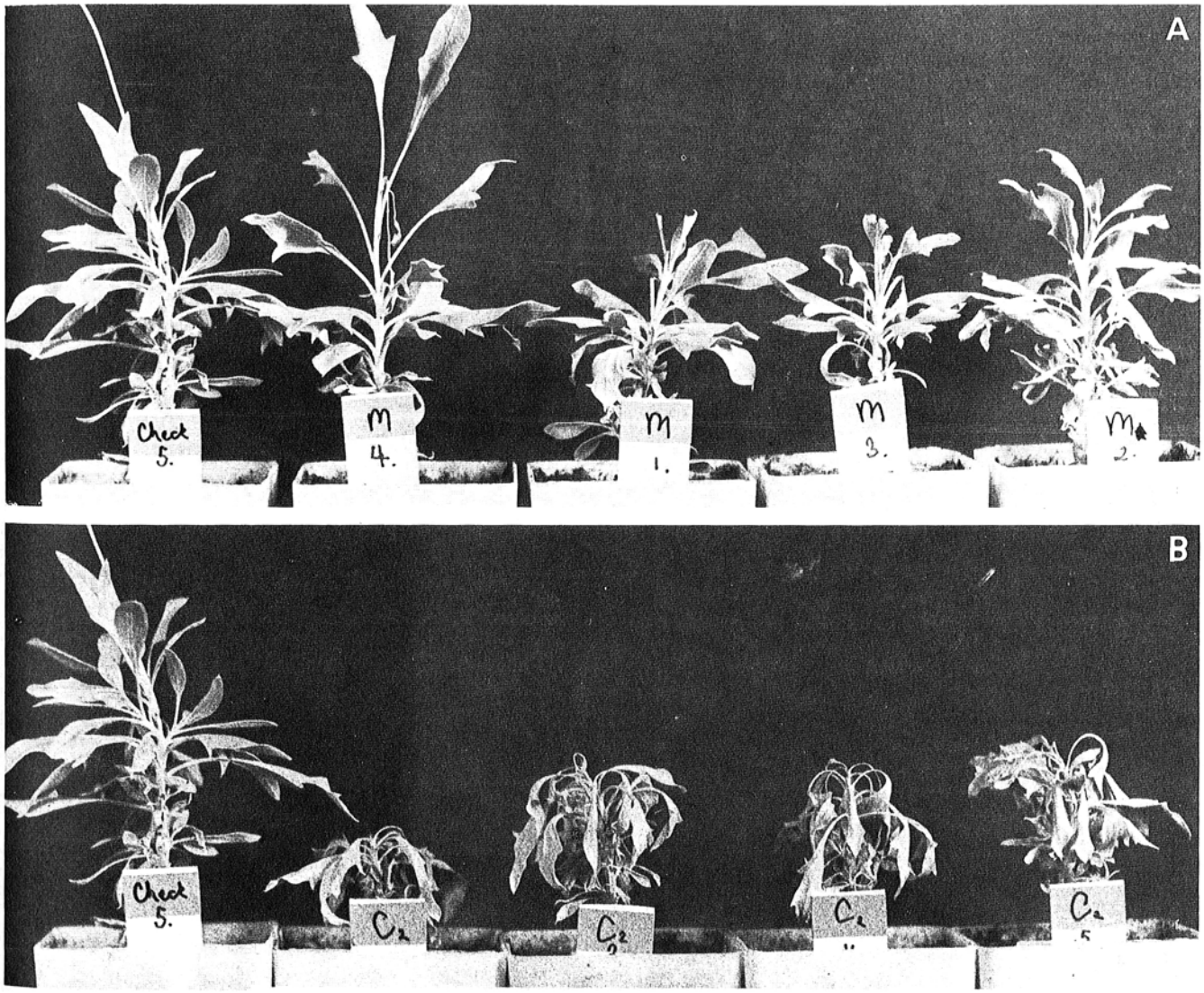


**Fig. 1.** Seedlings of guayule line LA-1 40 days after inoculation with strain M **(A)** and strain $C_2$ **(B)** of *Verticillium dahliae*.

instance (one out of five) from *P. tomentosum* var. *stramonium* and *P. confertum* var. *lyratum*, which also showed no symptom development.

## DISCUSSION

Of the two *V. dahliae* strains isolated from field-grown guayule at our test plot, $C_2$ was more virulent to guayule than $C_1$, even though their cultural characteristics on potato-dextrose agar and prune extract agar were similar. However, $C_2$ produced spores more abundantly than $C_1$. These two strains from guayule are different from the two strains (defoliating and nondefoliating) that are found on cotton. All of the eight strains of *V. dahliae* used in this study were pathogenic to guayule, particularly $C_2$, $E_{V3H}$, and $S_{T-1}$.

Gerstel (3) indicated that the diploid seedlings of guayule are more susceptible to Verticillium wilt than the tetraploid seedlings. He raised the possibility that resistance to Verticillium wilt may be related to chromosome number. Our results indicated an earlier development of wilt symptoms in the diploid than in the tetraploid seedlings. Tetraploids are woodier than diploids, and they may show higher tolerance to wilt development. This assumption may also apply to differences among various ages of guayule plants. Younger guayule seedlings developed and succumbed to wilt symptoms more rapidly than older ones (Table 3).

Other species of *Parthenium* showed high resistance, if not immunity, to $C_1$ and $C_2$ strains. The $C_1$ and $E_{V3H}$ strains were reisolated from inoculated, symptomless *P. fruticosum* plants. *P. fruticosum* was quite susceptible to the cotton-

**Table 2.** Effect of *Verticillium dahliae* strain on wilt severity in diploid seedlings (2N) and line LA-1 tetraploid seedlings (4N) of guayule[a]

| Strain | Guayule[b] line | Wilt index[c] after 13 days | 20 days | 27 days | 34 days | 40 days |
|---|---|---|---|---|---|---|
| M | 2N | 1.92 ± 1.16 | 3.50 ± 0.96 | 3.88 ± 0.68 | ...[d] | ... |
| | 4N | 0 | 0 | 1.50 ± 0.63 | 1.60 ± 0.80 | 1.60 ± 0.80 |
| F | 2N | 2.58 ± 0.95 | 3.83 ± 0.37 | 4.25 ± 0.75 | ... | ... |
| | 4N | 0 | 0 | 1.70 ± 0.60 | 2.50 ± 0.89 | 3.0 ± 0.70 |
| $S_{SS4}$ | 2N | 1.83 ± 1.21 | 3.75 ± 0.60 | 3.83 ± 1.48 | ... | ... |
| | 4N | 0 | 0 | 3.20 ± 0.81 | 5.0 ± 0 | 5.0 ± 0 |
| $E_{330}$ | 2N | ... | ... | ... | ... | ... |
| | 4N | 0 | 0 | 2.20 ± 1.46 | 2.70 ± 1.32 | 3.3 ± 0.97 |
| $S_{T-1}$ | 2N | 2.00 ± 1.29 | 3.58 ± 0.86 | 4.33 ± 0.77 | ... | ... |
| | 4N | 0 | 0 | 4.60 ± 0.48 | 5.0 ± 0 | 5.0 ± 0 |
| $E_{V3H}$ | 2N | 3.25 ± 1.09 | 3.17 ± 1.21 | 3.92 ± 1.35 | ... | ... |
| | 4N | 0 | 0 | 2.60 ± 1.39 | 3.0 ± 1.37 | 3.30 ± 1.60 |
| $C_1$ | 2N | 2.67 ± 1.03 | 3.75 ± 0.62 | 4.0 ± 0.74 | ... | ... |
| | 4N | 0 | 0 | 2.50 ± 1.0 | 4.0 ± 1.26 | 4.60 ± 0.48 |
| $C_2$ | 2N | 3.67 ± 0.62 | 4.0 ± 0 | 4.58 ± 0.34 | ... | ... |
| | 4N | 0 | 0 | 2.80 ± 0.24 | 5.0 ± 0 | 5.0 ± 0 |

[a] Twelve replicates for diploid and five replicates for tetraploid seedlings in two separate experiments.
[b] Four-month-old seedlings, each inoculated with 4 ml of spore suspension at $5 \times 10^7$ spores per milliliter.
[c] 0 = no wilt, 1 = 5–25% wilt, 2 = 25–50% wilt, 3 = 50–75% wilt, 4 = 75–95% wilt, and 5 = dead.
[d] Not recorded.


**Fig. 2.** Seedlings of *Parthenium fruticosum* 40 days after inoculation with strain $S_{SS-4}$ **(A)** and strain $S_{T-1}$ **(B)** of *Verticillium dahliae*.

**Table 3.** Effect of seedling age on wilt severity in guayule line LA-1 inoculated with *Verticillium dahliae* strain $C_2$[a]

| Age (mo) | Wilt index[b] after | | |
|---|---|---|---|
| | 23 days | 30 days | 44 days |
| 2 | 3.10 ± 1.50 | 5 ± 0 | 5 ± 0 |
| 3 | 2.55 ± 1.30 | 4.9 ± 0.30 | 5 ± 0 |
| 5 | 0.70 ± 0.64 | 1.75 ± 0.55 | 3.40 ± 1.41 |
| 7 | 0.45 ± 0.45 | 1.10 ± 0.50 | 4.45 ± 0.65 |
| 8 | 0.25 ± 0.25 | 1.18 ± 0.55 | 2.50 ± 1.0 |
| 18 | 0 | 0 | 0 |

[a] Ten replicates per plant with spore suspension at $5 \times 10^7$ spores per milliliter.
[b] 0 = no wilt, 1 = 5–25% wilt, 2 = 25–50% wilt, 3 = 50–75% wilt, 4 = 75–95% wilt, and 5 = dead.

defoliating strain $S_{T-1}$ (Fig. 2), but it was resistant to all other strains. *P. tomentosum* var. *stramonium*, *P. confertum* var. *lyratum*, and *P. incanum* were found to be resistant to the eight strains of *V. dahliae* tried. However, a few recoveries were positive from these symptomless plants: F strain from *P. tomentosum* var. *stramonium* and *P. confertum* var. *lyratum*, and $S_{T-1}$ from *P. incanum*. We thus assume that these *Parthenium* species could be susceptible to wilt induced by other strains of *Verticillium* besides the eight strains we have used in this investigation.


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